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Clostridial Enteric Diseases of Domestic Animals

J. GLENN SONGER*

Department of Veterinary Science, University of Arizona, Tucson, Arizona 85721

INTRODUCTION

Members of the genus *Clostridium* are widely recognized as enteric pathogens of humans, domestic animals, and wildlife (Tables 1 and 2). Their array of proven and putative virulence attributes is impressive, and infections take a plethora of forms in myriad hosts. In spite of the ready availability of inexpensive, usually effective products for immunoprophylaxis, clostridial enteric infections remain a common presentation at veterinary diagnostic laboratories.

This review will be limited to the more common clostridial enteric diseases of domestic animals, although reference will be made to human diseases as needed to provide a contextual background. Some infections that might be considered enteric (e.g., *Clostridium chauvoei* infection of gastrointestinal musculature and toxin production by *Clostridium botulinum* in the gut) are not addressed. Recent reviews (40, 142, 176, 261, 263, 264, 275, 292, 384, 388) may be useful in exploring other aspects of pathogenesis, diagnosis, and treatment.

CLOSTRIDIUM PERFRINGENS

Introduction

C. perfringens may be the most widely occurring pathogenic bacterium (384) and is certainly the most important cause of clostridial enteric disease in domestic animals (Table 1). Some types of *C. perfringens* (mainly type A) are consistently recovered both from the intestinal tracts of animals and from the environment, while others (types B, C, D, and E) are less common in the intestinal tracts of animals (68) and can occasionally be found in the environment in areas where disease produced by these organisms is enzootic (292). Because it is a frequent postmortem invader from the gut, isolation of *C. perfringens* from tissues of dead animals must be viewed with caution when making a diagnosis (69).

Major Toxins

As many as 17 exotoxins of *C. perfringens* have been described in the literature (163, 263, 266, 271, 353, 388), but a definitive role in pathogenesis has been demonstrated for only a few. The species is divided into types on the basis of production of the four major toxins, α, β, ε, and ι (Table 3), as determined by in vivo protection tests performed by intradermal injection of guinea pigs or intravenous (i.v.) inoculation of mice (47, 263, 387, 388, 434). Toxin (filtered culture supernatant fluids or eluates from gut contents), both trypsin treated and untreated, is injected alone or mixed with antiserum. Responses in guinea pigs (dermonecrosis) are noted after 24 and 48 h and in mice (lethality) through 72 h. Types are determined from the results, with type A defined as strains producing α toxin, type B as strains producing α, β, and ε toxins, type C as strains producing α and β toxins, type D as strains producing α and ε toxins, and type E as strains producing α and ι toxins (Table 1). Some strains of type A do not produce enough α toxin to kill mice under typical test conditions, and nonlethal strains are referred to as nontoxigenic by some but as type A by others (176). Based upon production of certain minor toxins, varieties or subtypes have been reported within types A, B, and C (47, 298), although some contend that establishing new types to distinguish minor differences would become unmanageable (397).

* Phone: (520) 621-2962. Fax: (520) 621-6366. Electronic mail address: gsonger@ccit.arizona.edu.

TABLE 1. Diseases produced by toxigenic types of *C. perfringens*[a]

Toxin type	Major toxin(s)	Diseases
A	α	Myonecrosis, food poisoning, necrotic enteritis in fowl, enterotoxemia in cattle and lambs, necrotizing enterocolitis in piglets; possibly equine colitis, canine hemorrhagic gastroenteritis
B	α, β, ε	Dysentery in newborn lambs, chronic enteritis in older lambs (pine), hemorrhagic enteritis in neonatal calves and foals, hemorrhagic enterotoxemia in adult sheep
C	α, β	Enteritis necroticans (pigbel) in humans; necrotic enteritis in fowl; hemorrhagic or necrotic enterotoxemia in neonatal pigs, lambs, calves, goats, foals; acute enterotoxemia (struck) in adult sheep
D	α, ε	Enterotoxemia in lambs (pulpy kidney) and calves, enterocolitis in neonatal and adult goats, possibly enterotoxemia in adult cattle
E	α, ι	Enterotoxemia likely in calves and lambs, enteritis in rabbits; host range and disease type unclear
A–E	Enterotoxin	Canine and porcine enteritis; possibly bovine and equine enteritis

[a] Data taken from references 250, 263, 292, 293, and 353.

The α toxin, the principal lethal toxin of *C. perfringens*, is a multifunctional phospholipase, produced in varying amounts by all (or nearly all) isolates. Hydrolysis of membrane phospholipids in erythrocytes, platelets, leukocytes, and endothelial and muscle cells results in lysis or other forms of cytotoxicity (102, 272, 388). It is hemolytic, necrotizing (408), and potently lethal (353). The α toxin gene, *cpa*, has been cloned and sequenced (237, 306, 359, 411, 419), and homologous genes have been found in other clostridia (410).

The β toxin is a highly trypsin-sensitive protein (200, 362) which is responsible for mucosal necrosis and possibly for central nervous system signs in *C. perfringens*-induced disease in domestic animals (200, 266). As little as 2 ng of β toxin produces dermonecrosis in guinea pigs, and the i.v. 50% lethal dose (LD_{50}) for mice is ≈500 ng/kg (362, 363, 448). Cloning and characterization of the β toxin gene (*cpb*) from a type B strain revealed homology with *Staphylococcus aureus* α toxin, γ toxin, and leukocidin (232, 445). *cpb* apparently resides on a large extrachromosomal element (77, 99). Southern blots and PCR analysis revealed that *cpb* is present only in *C. perfringens* strains of types B and D (164).

TABLE 3. Activities of the major toxins of *C. perfringens*

Toxin	Activity and effects
α	Phospholipase/sphingomyelinase C, hemolytic, lethal, necrotizing
β	Induces inflammation, necrosis of intestinal mucosa, lethal
ε	Protease-activated prototoxin; increases intestinal permeability; central nervous system toxicity; lethal
ι	Ia ADP-ribosylates actin; Ib mediates binding; dermonecrotic, lethal

The ε toxin is produced as the minimally toxic ε prototoxin (266), which is converted to the >1,000-fold-more-toxic form by proteolytic removal of 14 N-terminal amino acids (34). Although its precise biological activity has not been identified, ε toxin is necrotizing and lethal (19, 54), and its toxicity is estimated to be 3.2×10^6 i.v. minimum lethal doses per mg in mice (363). The ε toxin gene (*etx*) is thought to reside on a large plasmid, distinct from that bearing *cpb*, and is found only in strains of types B and D (77). The recent cloning and characterization of *etx* (181) will yield new information about toxin structure and function and provide the basis for second-generation vaccines (442).

The ι toxin consists of components Ia and Ib (65, 400, 401). Globular skeletal muscle and nonmuscle actin are ADP-ribosylated by Ia (380, 427), which is targeted to sensitive cells and enters the cytosol in a process mediated by Ib (79, 353, 355, 401). It increases vascular permeability (80) and is dermonecrotic and lethal for mice at higher doses (44). ι toxin shares attributes of structure and activity with the toxin of *Clostridium spiroforme* and with C2 toxin of *C. botulinum* types C and D (325) and is antigenically related to the former (331, 334) and to an ADP-ribosyltransferase in *Clostridium difficile* (333).

Enterotoxin (CPE) is the best understood of the major virulence attributes of *C. perfringens* (140, 141, 266, 270). Production of CPE is coregulated with sporulation; the toxin is released upon lysis of the vegetative cell. Proteolytic removal of 24 N-terminal amino acids from CPE activates the molecule, which then consists of a cytotoxic N-terminal domain and a C-terminal domain with receptor activity. The initial interaction of CPE with cells results in pore formation, followed by altered permeability, inhibition of macromolecular synthesis, cytoskeletal disintegration, and lysis (141, 156, 180, 257, 260, 262). Although traditionally associated with strains of type A, CPE and its gene (*cpe*) have been demonstrated in strains of other types (77, 393). *cpe* is a single chromosomal locus in some (perhaps most) strains, but recent findings suggest that it may also be found extrachromosomally, associated with a mobile genetic element (77, 141).

Disease and Pathogenesis by Toxin Type

In spite of the vast amount of circumstantial and indirect evidence for roles for various toxins and other molecules in the pathogenesis of *C. perfringens* infections, there is direct proof

TABLE 2. Enteric infections by clostridia other than *C. perfringens*

Organism	Major toxin(s)	Disease(s)
C. septicum	α	Abomasitis (braxy or bradsot) in sheep, possibly calves
C. difficile	A (enterotoxin), B (cytotoxin)	Pseudomembranous colitis (often antibiotic associated) in humans and laboratory animals, hemorrhagic necrotizing enterocolitis in foals, chronic diarrhea in dogs
C. spiroforme	ι toxin (ADP-ribosylating)	Diarrhea in weaned rabbits
C. colinum	None confirmed	Ulcerative enteritis (quail disease) in fowl

only for the role of the α and θ toxins in histotoxic infections (9). The promise of second-generation vaccines, perhaps based on recombinant toxins or toxoids, as well as advances in development of methods for genetic manipulation of *C. perfringens*, should provide direct evidence regarding the roles of the various toxins and other putative virulence attributes in the pathogenesis of enteric infections. Until that time, discussion of pathogenesis must, in large part, take the form of correlation of reported properties of virulence factors with clinical signs and lesions observed in affected animals. In this review, pathogenesis is discussed by toxin type and, to the extent possible, by specific disease syndrome.

Type A. Strains of type A (Table 1) are the most widespread in the intestines of warm-blooded animals and in the environment (408), being found in most soils that are specifically cultured for *C. perfringens* (384). Well known as causes of wound contamination, anaerobic cellulitis, and gas gangrene (163), strains of type A also cause enteric diseases. Enterotoxemia in lambs, known as yellow lamb disease, occurs primarily in spring in California and Oregon, when the population of nursing lambs is high (124, 267). Affected animals exhibit depression, anemia, icterus, and hemoglobinuria, dying after a clinical course of 6 to 12 h. Elevated counts of *C. perfringens* (10^4 to 10^7 CFU/g) are often found in intestinal contents. A similar condition has been reported in goats (356) and calves (137, 354). Type A strains have been isolated frequently in the western United States from calves with tympany, abomasitis, and abomasal ulceration. Gross lesions include hemorrhage and ulceration of the abomasal mucosa. Severe diarrhea may occur in calves, but enteric lesions can be obscured by rapid autolysis. Gram-positive bacilli are often found on the mucosa and in the submucosa (87, 89, 351). Some isolates from cases such as these contain *cpe* (393).

Necrotic enteritis, a disease of domestic chickens worldwide (10, 95, 218, 219, 229, 287, 416), is usually caused by *C. perfringens* type A (4, 11, 218, 219, 243, 296, 415) or type C (287, 378). Isolates of type D (234) and untypeable isolates (218, 219, 241) have been reported. Disease also occurs in at least some species of captive wild birds (405) and in turkeys (134). Mild forms of disease result mainly in decreased rates of weight gain (209). Depression, inappetence, anorexia, and diarrhea occur in some affected birds (10, 30, 166, 242, 287), but the course is usually short, with no signs observed and birds simply found dead. Necrosis of both jejunum and ileum can extend the entire width of the mucosa (282, 344), while acute catarrh without necrosis is more common in the lower intestine (344). Gram-positive bacilli are often detected on the lamina propria and attached to cellular debris.

C. perfringens type A is common in the intestinal tract of chicks (378), and soil, dust, and contaminated feed and litter have been implicated as sources of infection (97, 218, 219). The disease can be reproduced by raising chicks on litter on premises with a history of the disease (155, 256, 438) or by administration of contaminated feed (243, 415), cultures of *C. perfringens* (3, 5, 6, 314–316), or culture supernatant fluids (4, 6).

High-fiber litter can damage the intestinal mucosa, predisposing birds to development of necrotic enteritis (3), as can concurrent infection with coccidia, which is often observed (3, 109, 166, 287, 377). There are apparent dietary effects on the incidence of necrotic enteritis in chickens (45, 198, 210, 415), one of which may be to increase the numbers of *C. perfringens* in the intestinal tract (287). Intestinal flora may also affect the occurrence and severity of necrotic enteritis (128). Chicks inoculated with *Lactobacillus acidophilus* or *Enterococcus faecalis* and then challenged with *C. perfringens* rarely die, compared with a 50% mortality rate in germ-free chicks and no deaths in conventional birds. Furthermore, coculture of *C. perfringens* with chick intestinal contents resulted in suppression of α toxin production (128, 129).

The α and β toxins have been detected in feces and intestinal contents of chickens and other birds with necrotic enteritis (109, 218, 219) and are believed to be responsible for the intestinal mucosal necrosis that is characteristic of this disease (268). More α toxin may be produced by isolates from birds with necrotic enteritis than by isolates from normal birds (174). Lesions can be reproduced by administration of crude preparations of α toxin (4, 6, 296) to conventional (5) or germ-free (128) chicks. The lethal effects of challenge of germ-free chickens with α toxin can be neutralized by antiserum (128), and α antitoxin may play a role in protection of fowl against enteritis (284).

Intestinal clostridiosis in adult horses presents as profuse watery diarrhea with high mortality, and large numbers of type A organisms are demonstrable in the gut (439, 440). *C. perfringens* infection has also been described in horses with hemorrhagic cecitis and in horses dying peracutely without diarrhea (341), but a definite role for *C. perfringens* in colitis of horses remains to be proven. Lesions in a neonatal foal with hemorrhagic diarrhea included extensive subserosal hemorrhage, with marked, diffuse necrosis of the villous mucosa, as well as hyperemia and hemorrhage of the lamina propria, submucosa, and subserosa (56). Gram-positive bacilli were associated with necrotic villi. i.v. inoculation of ponies with a culture of type A caused acute colic and hemorrhagic enterocolitis (303).

Type A has been linked to enteric disease in suckling and feeder pigs with mild necrotizing enterocolitis and villous atrophy (78, 192–194, 309, 330). Lesions are usually most severe in the small intestine, particularly the jejunum and ileum (309), and culture of lesion material has frequently yielded heavy growth of *C. perfringens* (283, 309). A similar syndrome was produced by oral inoculation of gnotobiotic colostrum-deprived pigs as well as conventional weaner pigs (191). The results of experiments with α toxin in isolated loops of gut in pigs suggest that, alone, it may not produce significant lesions or fluid loss (112, 330).

Hemorrhagic canine gastroenteritis is sometimes associated with *C. perfringens*, often of unidentified type (336). Less severe forms present as watery-to-mucoid diarrhea with occasional blood, with ileal lesions similar to those in dogs with hemorrhagic enteritis and colitis. Peracute death is common, with hemorrhagic necrosis of the mucosa. Gram-positive bacilli are common in and on necrotic tissue but apparently do not invade normal tissue (52, 57, 223, 336).

Other reports of enteric disease associated with *C. perfringens* type A include enterotoxemia in minks (248), muskrats (432), and racing camels (374), gastroenteritis in black-footed ferrets (372), and acute toxemia in water buffaloes (447).

Although little is known of the permeability of the intestine to α toxin, it may be a virulence factor in cases of presumed enterotoxemia (87, 89). In some cases, the syndrome in lambs and calves is consistent with the action of a hemolytic toxin in the circulation, causing massive intravascular hemolysis and capillary damage, inflammation, platelet aggregation, shock, and cardiac effects, culminating in death (399, 408). Large amounts of α toxin can be found in feces in natural cases of disease in cattle. Studies in neonatal pigs revealed that α-toxigenic *C. perfringens* could cause enteropathy after substantial multiplication in the gut, and proliferation was accompanied by adherence or invasion (192–194). On the other hand, α toxin administered alone to piglets 1 to 6 h old caused mild

enteritis and villous edema, with minimal damage to the epithelium and blood vessels, but no ultrastructural changes in villi, lymphatics, or other tissues (192–194). i.v. injection of lambs (299) or calves (295) with α toxin produced transitory diarrhea and intestinal mucosal hyperemia. Recent evidence suggests that minor differences in the amino acid sequence of α toxin from gas gangrene-causing strains (possibly possessing a predilection for muscle tissue) and enterotoxemia-causing strains (possibly capable of multiplication and disease production in the gut) confer increased resistance to chymotrypsin on toxin from the latter strains (137), perhaps allowing accumulation in the gut with subsequent entry into the circulation.

A central role for α toxin has been demonstrated in pathogenesis of muscle disease (9, 49, 50), and α antitoxin is protective against such infections (441). Antibodies against the genetically truncated N-terminal portion of α toxin (amino acids 1 to 249) neutralized phospholipase C but not hemolytic activity, and animals immunized with these truncated proteins were not protected against challenge with toxin or with *C. perfringens*. On the other hand, antibodies against the C-terminal portion of the molecule (amino acids 247 to 370) neutralized both phospholipase C and hemolytic activities, and immunization with this peptide protected mice against challenge with toxin or with 10 LD_{50}s of *C. perfringens* (409, 441). These findings suggest the need for further study of the role of α toxin in enteric disease in domestic animals, including evaluation of the efficacy of currently available commercial toxoids in producing immunity against α toxin action in the gut.

Type B. *C. perfringens* type B is the etiologic agent of dysentery in newborn lambs (Table 1). Disease occurs primarily in the border country between England and Scotland, as well as in Wales, South Africa, and the Middle East (408). A similar disease was reported in Montana (420), although isolates of type B are relatively rare in North America. Lamb dysentery usually develops during the first few days of life, although older lambs may be involved as an outbreak progresses (85). After the infection is acquired from the dam or the environment, organisms in the gut increase in numbers, especially with heavy lactation by the dam. The result is enterotoxemia accompanied by enteritis and extensive hemorrhage and ulceration of the small intestine (125, 350). The primary sign is sudden death, without forewarning in peracute cases. In acute incidents, cessation of feeding and severe abdominal pain are accompanied by bloody diarrhea, with recumbency, coma, and death less than 24 h after onset. Incidence is often as high as 30%, with case fatality rates approaching 100%. Chronic disease (called pine) in older lambs manifests as chronic abdominal pain without diarrhea. Type B may also be associated with hemorrhagic enteritis in goats, calves, and foals (125, 350, 402).

There has been little experimental study of the pathogenesis of infections by type B (188). It is not known whether an individual effect of α, β, or ε toxin predominates or if there are additive or synergistic effects. Recent advances in genetic manipulation of *C. perfringens* may allow the generation of appropriate mutants to experimentally answer these and other questions.

Type C. Infections by *C. perfringens* type C have been reported in pigs, cattle, sheep, horses, chickens, humans, and dogs in the United States, Denmark, Great Britain, Japan, and elsewhere (249) (Table 1). Newborn animals are typically most susceptible, perhaps because of ready colonization of the gut by *C. perfringens* in the absence of well-established normal intestinal flora. Alteration of the flora by sudden dietary changes may also be an inciting factor in type C infections (408).

Piglets are perhaps more commonly affected by type C than are other domestic animals (123, 199, 313, 407). Peracute disease may affect piglets 1 to 2 days of age (249, 294). The attack rate varies among herds and litters (294, 300), and once established in a herd, disease often becomes endemic. Morbidity rates of 30 to 50% are not uncommon (294, 305, 307), with case fatality rates of 50 to 100%, often with a clinical course lasting less than 24 h. Depression is followed by diarrhea and dysentery, with blood and necrotic debris in feces. Piglets affected at 1 to 2 weeks of age often have a more protracted clinical course, with nonbloody, yellowish diarrhea and necrosis of the jejunal mucosa (249, 294). In younger piglets, the intestinal surface is often dark red, with gas accumulation in tissue and hemorrhagic exudate in the lumen; hemorrhagic necrosis of the mucosa, submucosa, and even muscularis mucosa is usually extensive (7, 75, 116, 249, 305, 307, 428). As many as 10^9 CFU of *C. perfringens* can be isolated per g of intestinal contents, as either mixed cultures of types A and C (305, 393) or nearly pure cultures of type C (274). Although sows are thought to be a common source of the infection for newborn pigs, numbers in sow feces may be too low to be detected by culture (305).

A similar disease occurs with type C in neonatal calves (145, 301), lambs (146), and goats. Vigorous, healthy calves, usually less than 10 days of age, develop hemorrhagic, necrotic enteritis and enterotoxemia, often accompanied by evidence of abdominal pain, frenzied bellowing, and aimless running; in lambs, the disease resembles lamb dysentery (288, 412). Nervous signs, including tetany and opisthotonus, may also occur. Death may be peracute, occasionally without other clinical signs, but may also follow a clinical course of several days. Attack rates often reach 15 to 20%, with case fatality rates of 100%.

Adult sheep (typically young ewes) in certain geographic locales (Wales and the Romney Marsh areas of England) can be affected by an enterotoxemia colorfully named struck. The name derives from the rapid death associated with the condition, which often leaves the impression that the animal has been struck by lightning. In areas where it is endemic, the organism is probably widespread in soil, with most animals being infected (408). Following damage to the mucosa of the gastrointestinal tract, often caused by poor-quality feed, type C multiplies in the abomasum and small intestine, causing mucosal necrosis, usually without dysentery or diarrhea. Evidence of toxemia may include accumulation of fluid in the peritoneum and thoracic cavity, and visible lesions are sometimes absent from the gut (396).

Type C is the most commonly reported clostridial enteric pathogen of foals in North America (96, 98, 179, 317, 381). Clinical signs include depression, severe hemorrhagic diarrhea, dehydration, and, occasionally, colic (179). Lesions in the jejunum and ileum usually consist of acute hemorrhagic enteritis with necrosis of villi and large numbers of gram-positive rods demonstrable in stained smears and sections.

At least one type C isolate has been obtained from peracute-to-acute, lethal, hemorrhagic enteritis in dogs (8, 226). Untyped isolates, associated with a similar but milder necrotic process, were also obtained from a high proportion of dogs with a primary parvovirus infection (421). In the latter case, gram-positive bacilli were observed in necrotic areas of the small intestine.

Studies of β toxin have focused mainly on susceptibility of hosts, clinical disease, histopathology, and pharmacology, with little study to date of the molecular mode of action. β toxin causes increased capillary permeability, perhaps facilitating its uptake from the gut to the circulation and promoting subsequent systemic effects. i.v. administration of purified β toxin is followed by an increase in blood pressure and decreased heart

rate, with electrocardiographic disturbances suggestive of atrioventricular block (364). The similarities of *cpb* to the genes for staphylococcal α and γ toxins and leucocidin (182) strengthen suggestions that β toxin may affect the central nervous system (33, 200, 266, 445).

It is widely accepted that β toxin plays a central role in the pathogenesis of type C infections. In pigs, *C. perfringens* adheres to the jejunal mucosa (7), although ultrastructural studies revealed damage to microvilli, mitochondria, and terminal capillaries prior to bacterial adhesion (195–197). Events initiated at the apices of the jejunal villi progress to widespread mucosal necrosis, and gram-positive bacilli, as well as spores (224), may be demonstrated in stained smears or sections across vast areas of the mucosa. Mucosal necrosis is progressive, with epithelial cell death and desquamation followed by further bacterial invasion, multiplication, and more toxin production.

The initial action of β toxin in the jejunum is under conditions of curtailed proteolytic activity. This may be due to a pancreatic secretion deficiency during a short period after birth or to the ingestion of protease inhibitors, which compromise the ability of the gut to detoxify β toxin (101, 294). A trypsin inhibitor in colostrum may contribute to the pathogenesis of type C infections in piglets (293). Although intestinal lesions can be impressive in extent and severity, death is probably due ultimately to β toxemia (47, 224, 294). Acute or peracute deaths are common (105), and if animals display clinical signs (such as diarrhea), it is usually for only a brief period before death.

In spite of the probable central role of β toxin, typical disease cannot be reproduced by use of toxin alone. Experimental reproduction of the disease in pigs requires viable bacteria as well as toxin (as crude culture supernatant fluids) (23, 294). Reproduction of acute (ultimately fatal) hemorrhagic enterotoxemia in lambs was achieved by inoculation with cultures of type C or with cells resuspended in fresh medium (both in the presence of soybean flour as a protease inhibitor) but not with cell-free culture supernatant fluid (297). The natural history of the disease in the field suggests that only organisms are capable of initiating the infectious process. The cloning and characterization of *cpb* (182), as well as the development of methods for genetic manipulation of pathogenic clostridia, may lead to engineering of Cpb^- mutants of type B or C organisms, allowing elucidation of the role of β toxin in pathogenesis.

Type D. Type D strains (Table 1) cause enterotoxemia, also known as sudden death or overeating, in sheep of all ages except newborns (408). It is probably most prevalent in lambs 3 to 10 weeks old, often suckling heavily lactating ewes grazed on luxuriant pastures. Enterotoxemia is also a predominant cause of death in weaned animals up to 10 months of age, usually those fed rich rations of grain in feedlots. Disease is often associated with upsets in the gut flora, such as result from sudden changes to a rich diet or from continuous feeding of high levels of feed concentrates (332). Appropriate microenvironments lead to rapid multiplication of type D and production of ε toxin, almost certainly favored by the presence of excess dietary starch in the small intestine (51). A high concentration of ε toxin facilitates its absorption (293). A true toxemia results, with little evidence of enteritis, and the effects of ε toxin on the central nervous system and other tissues cause sudden death, with some animals surviving long enough to display clinical signs such as dullness, retraction of the head, opisthotonus, and convulsions, with agonal struggling (293, 332). Focal encephalomalacia occurs sporadically in affected sheep and is characterized by haphazard roaming, blindness, head pressing, and inability to eat. It is likely a chronic neurological manifestation of enterotoxemia, identified by the occurrence of bilaterally symmetrical brain lesions (56, 161). Pulpy kidney, another common name for type D enterotoxemia, is derived from one of the hallmark lesions in affected sheep, a result of postmortem autolysis, which occurs rapidly in hyperemic, toxin-damaged tissue.

Type D enterotoxemia is also important in calves (148, 281), in goats (312, 431), and rarely in adult cattle (280), deer, domesticated camels, and horses (67, 402). Enterotoxemia in suckling calves is similar to the disease in sheep (148, 245). In neonatal and adult goats, however, catarrhal, fibrinous, or hemorrhagic enterocolitis is a consistent lesion (35, 36, 431), and the classic pulpy kidney is absent.

Small amounts of ε toxin detected in the gut of normal animals are considered innocuous, but persistence of high concentrations of ε toxin leads to an increase in permeability and to absorption of toxin into the circulation (51). A primary target of ε toxin is the central nervous system, where it produces foci of liquefactive necrosis, perivascular edema, and hemorrhage, especially in the meninges (55). A receptor for ε toxin has been identified on vascular endothelial cells, and a high-affinity binding site for toxin may be a sialoglycoprotein in synaptosomal membranes (285). Ultrastructural examination of brain tissue from animals inoculated with ε toxin revealed that tight junctions in the vascular endothelium degenerate (53), causing perivascular astrocyte processes to swell and rupture (121). This leads to an increase in capillary permeability (120), with loss of plasma proteins (119) and water and rapid extravasation of fluid, and is followed by elevated intracerebral pressure (53). Focal-to-diffuse areas of degeneration and necrosis develop (53), and bilateral macroscopic foci of encephalomalacia can occur (56, 120, 121, 161). The extent of clinical signs of central nervous system derangement, including incoordination and convulsions, is directly related to the severity of lesions (147).

Hemorrhage is not a hallmark of typical type D-induced disease in sheep or cattle, although hemorrhagic areas in the small intestine and petechial hemorrhages of the endocardium can be present, as can subendocardial hemorrhage around the mitral valve (206). Disease in goats frequently presents as hemorrhagic enterocolitis, which can be chronic; it is often associated with lactation and high food intake. The basis for this species-to-species difference in clinical picture is unknown (36). Peritoneal and pericardial effusions are typical in sheep (206). Hyperglycemia and glycosuria are pathognomonic for type D enterotoxemia (132, 293). These events apparently occur very rapidly, probably in the first hour after ε toxin enters the circulation.

ε toxin stimulates the production of specific antibody in infected animals, even those with subclinical disease. Neutralizing monoclonal antibodies have been produced, as have anti-idiotypic monoclonal antibodies (323), suggesting that neutralization of toxin can be achieved at a single epitope.

Type E. Type E is an apparently uncommon cause of enterotoxemia of lambs, calves, and rabbits (16) (Table 1). ι enterotoxemia in a calf and lambs was reported 50 years ago in Britain, but it is uncertain if published accounts since that time (of hemorrhagic, necrotic enteritis of calves in Australia [160] and of detection of the organism and ι toxin in ovine or bovine intestines postmortem in the absence of signs of clostridial enterotoxemia [396]) represent genuine cases. Recent experimental work demonstrated the ADP-ribosylating nature of ι toxin, and characterization of the genes will provide a further basis for study of this toxin's role in the pathogenesis of intestinal infections. The functional similarity of *C. perfringens* ι toxin to the toxin of *C. spiroforme* suggests that infection by the

latter may be mistaken for infection by the former and vice versa if detection of toxin in the gut is the sole diagnostic measure. Further experimental work is needed to clarify the role of each of these organisms as etiologic agents of disease in domestic animals.

Enterotoxigenic *C. perfringens*. Production of CPE by strains of type A (and other types as well) complicates the interpretation of results in some reports of type A clostridial enteric disease (Table 1). In many cases, investigators have not examined clinical materials for CPE, nor have isolates been surveyed for CPE production. Determination of a distinct role for enterotoxigenic *C. perfringens*, regardless of toxin type, in enteric disease of domestic animals might dispel much of the confusion that now surrounds interpretation of etiology and pathogenesis.

In fact, an increasing body of evidence suggests a role for enterotoxigenic strains of type A in the etiology of diarrheal conditions in several animal species (110, 292, 293). Enterotoxigenic strains have been detected in many species of animals and in the environment (423–425). In one study, 6% of isolates from food handlers were enterotoxigenic, as were 2% from dogs, 12% from oysters, and 10% from water (361). In another study, *cpe* was detected by DNA hybridization in 14% of samples from horses, 22% from cattle, and 10% from poultry (418), and toxin production was observed in 12% of isolates from cattle, sheep, and chickens with enteritis (296). Genotyping studies with PCR assays used to detect *cpa*, *cpb*, *cpe*, and *etx* revealed that 3.1% of type A isolates were enterotoxigenic, compared with no type B or C isolates and 8.8% of type D isolates (392, 393). Epidemiologic evidence suggests a possible animal-to-human route of transmission of CPE-producing strains of *C. perfringens* (41).

Enterotoxigenic *C. perfringens* as a cause of enteropathy in dogs has been a subject of interest (57, 223, 437), although little information on pathogenesis exists. These organisms have been associated with hospital-acquired diarrhea in dogs (223), with clinical signs including mild depression, anorexia, and soft-to-watery diarrhea, in some cases with blood and mucus. Diarrheic dogs had significantly higher numbers of *C. perfringens* in their feces (223), and CPE was detected in the feces of 41% of diarrheic dogs but in only 7% of normal dogs (52, 57, 223, 336). Sixty-five percent of isolates from dogs with fecal CPE also produced CPE in vitro (223).

Differences in the numbers of *C. perfringens* type A in the intestines of healthy horses and horses with intestinal disease (439) and the presence of CPE in the intestinal contents of diseased foals suggest that disease may result from infection with these *C. perfringens* strains (211, 394). Diarrhea and death have been reproduced by challenge with broth cultures of *C. perfringens* (440), and ponies inoculated with CPE developed colic and hemorrhagic gastroenteritis (211). The involvement of CPE in the pathogenesis of colitis X has been suggested, based on observations of large numbers of enterotoxigenic *C. perfringens* in the gut of affected horses (341, 371) and lesions produced in experimental infections with type A (335).

Enterotoxigenic *C. perfringens* has also been implicated in diarrheal disease in pigs. CPE is frequently found in stools of diarrheic pigs but is absent from matched normal animals (78, 110, 189–191, 283, 330, 406). Postmortem findings in naturally occurring disease included superficial mucosal necrosis and villous atrophy (78, 283). Among 42 type A isolates from diarrheic pigs, 13 produced spores at a frequency of $\geq$10%, and CPE production by 11 of these ranged in titer from 1:2 to 1:64, as determined by a cytotoxicity assay in vitro. Pigs with enteritis had spore counts of up to 5×10^6 CFU/g of feces or intestinal contents, while up to 2×10^4 CFU/g were found in nondiarrheic pigs (110).

Most of our knowledge about the pathogenetic role of enterotoxin is derived from study of disease in humans (171). Organisms ingested in food sporulate in the gastrointestinal tract. The toxin, released upon lysis of vegetative cells, is a single polypeptide of 35 kDa (320 amino acid residues), with an amino acid sequence unique among the bacterial toxins described to date. Its action on the intestinal mucosa, mainly in the jejunum and ileum, is rapid, with profuse diarrhea and associated clinical signs appearing in 5 to 30 min. The mode of action occurs in multiple steps, including binding to a specific receptor on cell membranes, insertion into the plasma membrane, and complexing with membrane proteins (one toxin molecule and membrane proteins of 70 and 50 kDa, for a final complex of 160 kDa [446]). The toxin apparently does not enter the cytoplasm, but its presence in the plasma membrane induces a rapid decrease in intracellular concentrations of ions and small molecules. Other effects include inhibition of synthesis of macromolecules, decreased energy metabolism, increased secretory flux, inhibition of glucose uptake, and outpouring of water, sodium, and chloride (265), manifested clinically as diarrhea.

The *cpe* gene has been cloned and sequenced (83, 185, 426). Mapping of functional regions of the molecule (157) revealed at least five distinct epitopes; the region responsible for receptor binding is located near the C terminus, the region encompassing amino acids 26 and 171 is implicated in insertion and cytotoxicity, and removal of the first 25 N-terminal residues increases cytotoxicity.

Inoculation of hysterectomy-derived, colostrum-deprived (HDCD) piglets with enterotoxigenic *C. perfringens* cells resulted in disease ranging from profuse, blood-stained diarrhea, enteritis, and death (112, 309) to creamy diarrhea, emaciation, and gas accumulation in the intestine, with low mortality, and more closely resembling natural and experimental infections of conventionally weaned pigs (309). Enterotoxin purified from pig feces or from human isolates of *C. perfringens* caused fluid accumulation in porcine ileal loops. Transient diarrhea followed intragastric administration of CPE to HDCD piglets (112, 330). When pregnant sows were immunized with a toxoid of CPE, their suckling pigs were specifically protected against oral challenge with an enterotoxigenic strain. Diarrhea and death were prevented in experimentally infected HDCD pigs by administration of serum, milk, or colostrum from sows immunized with the crude toxoid (111, 112).

Enterotoxin is weakly immunogenic when exposure is through the intestinal tract, and clinical disease gives rise to detectable serum antibodies in humans, pigs, sheep, cattle, and horses (110, 112, 291). Antibodies produced in response to parenteral inoculation are not protective. However, mouse antibodies to a conjugate of the 30 C-terminal amino acids of CPE and a thyroglobulin carrier neutralized native CPE toxicity, suggesting possible utility in vaccine development. Recent evidence suggests that the best target for immunoprophylaxis may be the initial membrane-binding event (157, 270). It may be that future commercial products for immunization of pigs against type A diarrhea should include CPE toxoid (111).

Reports of untyped *C. perfringens*. A noteworthy feature of many literature reports of *C. perfringens* enteric disease is the absence of information on the toxin type of the infecting organism. Included are rare milk-borne disease in humans (131); enterotoxemia in camels (108); enteritis in rabbits (324, 435), cattle (365), and wild elk (389); and diseases of captive felines (375).

Prophylaxis and Therapy

C. perfringens is commonly susceptible to penicillin G, cephalosporins, tetracyclines, chloramphenicol, avilamycin, salinomycin, monensin, furazolidone, rifampin, bacitracin, carbadox, erythromycin, lincomycin, clindamycin, amprolium, nitrovin, and virginiamycin but is resistant to flavomycin and usually to aminoglycosides (48, 72, 73, 91, 94, 135, 155, 173, 215, 221, 243, 256, 337). Prophylaxis in swine by use of bacitracin has been effective (373), and in chickens, virginiamycin, nitrovin, tylosin, penicillin, ampicillin, bacitracin, furazolidone, and efrotomycin have apparently been useful in limiting shedding (336, 383, 403, 404), although quantitative data are evidently not available.

Acquired resistance to tetracycline, bacitracin, virginiamycin, macrolide-lincosamide antibiotics, and other antimicrobial agents used for growth promotion and disease prophylaxis in strains isolated from swine and poultry has been reported (88, 94, 100, 352), and in one study (343), a majority of isolates from domestic animals were reportedly resistant to streptomycin, erythromycin, cephalosporin, lincomycin, tetracycline, bacitracin, and carbenicillin. Most reports suggest that penicillin-resistant strains do not exist (100, 352), although there is at least one report (343) that more than 15% of isolates are penicillin resistant. The results of studies of antimicrobial susceptibility should be interpreted with caution if the methods are not clearly presented and especially if appropriate control strains are not included. The nature of resistance of some strains of *C. perfringens* to tetracycline (352), chloramphenicol, and erythromycin has been reviewed recently (353).

In individual animals, therapy with equine hyperimmune antiserum may be of value (349), although death often occurs too rapidly for it to be applied. Passive immunization protects for 2 to 3 weeks.

Since the course of many *C. perfringens*-associated enteric diseases is rapid and frequently fatal, immunoprophylaxis is a control measure of paramount importance. Commercial vaccines are often multivalent, typically consisting of inactivated cells, toxins, or both (170, 433); there is unresolved debate regarding the primacy of antitoxic or antibacterial immunity. Semiannual booster injections are recommended by manufacturers, suggesting that immunity may last for 6 to 12 months. Products are available for protection of piglets (by immunization of sows during gestation) (175, 255, 349), and 10-fold reductions in mortality as a result of vaccination have been reported (422). Vaccination of the dam is ordinarily followed in about 2 weeks by protective levels of antibodies in colostrum (341). Although no commercial products are currently available, immunization of pregnant sows with an enterotoxin-containing toxoid protected suckling pigs from oral challenge with an enterotoxigenic isolate of *C. perfringens* from a piglet with enteritis. Control animals (infected and nonvaccinated) often died, emphasizing the potential importance of including enterotoxin in bacterin-toxoids for pigs (110).

Ewes may be vaccinated against infection by type B, C, or D, depending on the geographic locale. As with neonatal pigs, lambs are protected against dysentery by colostral antibodies (214, 304, 385) and may be immunized with bacterin as early as 3 days of age (214). Although the effectiveness of vaccination in sheep is good, it is not complete. The anti-ε toxin titer in goats following vaccination is similar to that in sheep (36, 143); although it prevents death from toxemia, it provides little protection against development of enterocolitis (35, 36).

Diagnosis

Several treatises describe detailed diagnostic procedures for clostridial diseases (68, 69, 434). The key components in diagnostic systems for enteritis caused by *C. perfringens* in domestic animals remain evaluation of clinical signs and gross and microscopic lesions, bacteriologic culture of appropriate specimens, and detection of toxins in pathologic specimens and in supernatant fluids of pure cultures (172, 386). Selective media may be employed (84). Isolation from humans has been achieved by capture of organisms on silicate beads coated with specific antibody to type C (231). A cautionary note is that *C. perfringens* strains that originate in the gut are present in the tissues of most cadavers within a few hours of death. Thus, the results of bacteriologic culture must be interpreted with caution if appropriate clinical signs and lesions are absent (408). Quantitation of *C. perfringens* from the gut has been claimed to be an indicator of disease occurrence in animals (86, 408, 439), and large numbers of spores in the gut may be a useful finding in diagnosis of enterotoxin-induced diarrhea; however, there is apparently little definitive evidence to support this contention (396). The numbers of *C. perfringens* in the normal gut apparently vary, but individual diagnosticians who have extensive experience may find such information useful as one part of a diagnostic armada. This is an aspect of clostridial diagnosis that could benefit from systematic study.

Demonstration of major toxins in the gut, the bloodstream, or serous exudates (69, 408) by in vivo assays has become less common because of the expense, variability of results, and undesirability, on humanitarian grounds, of the traditional mouse and guinea pig assays. An alternative method is based on the pattern of inhibition of hemolytic activity of field strains by type A and type C antisera (298), perhaps indicative of production of minor hemolysins. Cytotoxicity assays for enterotoxin have also been reported (32, 110, 251). Most other in vitro methods for detection of toxinogenic *C. perfringens* are based on enzyme immunoassays for detection of toxins or gene probes or PCR for detection of toxin genes. Enterotoxin has been detected by immunoassays, including enzyme-linked immunosorbent assay (ELISA) and reverse passive latex agglutination (15, 32, 81, 136, 158, 182, 186, 187, 258, 259, 269, 289, 302, 444). Recently, there have been several reports of immunoassays for enterotoxemia-associated toxins of *C. perfringens*, including ε toxin detection by immunoelectrophoresis (167, 225, 414), latex agglutination (252), immunodiffusion (21), and ELISA (103–105, 177, 253, 286, 290, 390). Production of monoclonal antibodies (37) led to development of a competitive ELISA for detection of ε antitoxin in rabbit serum (390).

An ELISA allowed detection of as little as 0.005 U of α toxin per ml of culture in cooked-meat medium (177). ELISA has also been demonstrated to detect 1 to 8 ng of β toxin per ml in purified form or from pure cultures (103, 286) and 30 ng/g from the gut of infected animals (104). The sensitivity and specificity range from 90% (104) to 100% (273) in comparison to mouse protection tests (253, 290). Detection of about 2 ng of ε toxin per ml of purified material (103), 0.1 ng/ml of pure culture (286), and 4 ng/g from the gut of infected animals was possible with another ELISA (104). The sensitivity and specificity in comparison to the mouse protection test were 95 to 98% (104, 290). ι toxin can also be detected in pure cultures by use of an ELISA (286). A similar assay has been applied to assessment of the potency of clostridial toxoids (37, 390). A latex agglutination test for ε toxin is simpler to perform than ELISA but lacks the sensitivity and specificity of ELISA (252).

Detection of one of the major toxins of *C. perfringens* in clinical specimens can be a useful diagnostic finding, but it does

not necessarily confirm the existence of disease, especially in the case of ε toxin, which can be found in the gut of apparently normal animals (396). Failure to demonstrate toxins by an in vivo assay, on the other hand (particularly β toxin in gut contents), may be insignificant because of the lability of these proteins in the presence of proteases.

The use of gene probes and PCR assays for detection of toxigenic *C. perfringens* in affected animals has also been reported (87, 89, 113–115, 164, 207, 220, 360, 418). These methods can be especially useful in determining the ability or potential ability of an isolate to produce CPE, since they do not require maintenance of cell cultures for toxin assays, and isolates that have *cpe* but do not sporulate in vitro can be readily detected (reducing or eliminating false negatives). Several groups have reported methods for detection of *cpe* by use of both oligonucleotide probes (87, 89, 418, 425) and PCR (114, 115, 207, 220, 360). As many as 22% of some species of domestic animals carried *cpe*-positive *C. perfringens*, as determined by colony hybridization methods (418). Six of 98 strains of *C. perfringens* from feces of farm animals contained *cpe*, based on screening with an oligonucleotide probe (424, 425). Further study of 245 food and fecal isolates from nearly 200 outbreaks of food poisoning revealed that 59% were *cpe* positive by hybridization. Detection of *cpe* by hybridization was thought to be more reliable than detection of CPE by an enzyme immunoassay for diagnosis of enterotoxin-associated disease (308, 425). Examination of specimens by oligonucleotide hybridization apparently ruled out enterotoxin in an outbreak of diarrhea in pigs (424). In another study, genes for the α, β, ε, ι, θ, and μ toxins as well as enterotoxin and sialidase were detected by colony DNA-DNA hybridization. Of more than 750 strains from cases of bovine enterotoxemia in Belgium, all hybridized with probes for the α toxin and sialidase genes, most hybridized with probes for the θ and μ toxin genes, a few hybridized with the probe for *cpe*, and none hybridized with probes for the β, ε, and ι toxin genes (89).

PCR assays also have the potential to simplify the process of diagnosing *C. perfringens*-induced enteric disease and typing of isolates. These methods are both sensitive and specific in predicting CPE production by individual isolates (220, 360). *cpe* could be detected in purified chromosomal DNA from enterotoxin-producing isolates as well as in supernatant fluids of cultures in a sporulation medium. The gene was detected in feces by heat shocking the specimen, culturing it in sporulation medium, and subsequently examining culture supernatant fluids by PCR. Good correlation was found between the results of bacteriologic culture and assays for *cpe* and CPE (360). Immunoblotting for CPE detection was compared with a PCR assay and a digoxigenin-labeled probe assay (220). *cpe* was detected dependably in DNA from strains of *C. perfringens*, and most produced CPE, as determined by immunoblotting. Those strains that were Cpe^- did not sporulate in vitro. Until more is known about in vivo sporulation of isolates that do not sporulate in vitro, gene detection assays may be preferable to serologic assays (220).

Primers derived from the sequences of *cpa*, *cpb*, *etx*, *i*A, and *i*B were successfully used in PCR assays for the toxin genes (114, 115, 207, 273, 391, 392). In one study (391, 392), amplification of the template from 491 isolates allowed them to be readily placed into genotype A (86.7%), genotype C (4%), and genotype D (9.3%). A multiplex PCR system for genotyping produced similar results (87). Others found that strains of types B and D were readily differentiated by PCR from strains of types A, C, and E without the use of toxicity neutralization tests (164). Detection of *C. perfringens* by amplification of *cpa* has also been reported (113), with a detection limit of $\approx 5 \times 10^2$ bacteria per g of feces. PCR genotyping methods have also been adapted to detection of the organism in direct enrichments of intestinal contents of affected animals (87, 273).

Continued refinement of immunoassays and methods for gene detection, including definition of comprehensive methods which minimize false positives and false negatives and development of commercial sources for appropriate diagnostic reagents, will simplify and increase the accuracy of diagnostic approaches to *C. perfringens*-induced enteric disease.

CLOSTRIDIUM SEPTICUM

Introduction

C. septicum, sometimes referred to as the malignant edema bacillus, grows readily under anaerobic conditions and is hemolytic. The G+C content of its DNA is 24 mol% (71). Commonly found in soil, *C. septicum* has also been isolated from the feces of domestic animals and humans (118, 208, 338, 340). It is a frequent postmortem invader from the gut of domestic animals, particularly ruminants (408). There is evidence that *C. septicum* can enter animal hosts from the environment in one of the life stages of liver flukes (133, 328); the organism was isolated from snails, which play a role in the life cycle of flukes, as well as from flukes recovered from sheep experimentally infected with the parasites. *C. septicum* was isolated from necrotic livers of three sheep that died after these experimental infections (328). Iatrogenic infections have also been reported (162, 278, 429) and seem to be more common in horses than in other species.

Virulence Attributes and Pathogenesis of Enteric Disease

Many aspects of *C. septicum*-induced nonenteric disease in domestic animals have been reviewed elsewhere (23, 150, 408). Wound infections by *C. septicum* in animals are generally referred to as malignant edema and usually follow direct contamination of a traumatic wound (250). In addition to its role in clostridial myonecrosis, *C. septicum* may also cause enteric infections (144, 370) (Table 2). In lambs and older sheep, the organism may penetrate the abomasal lining and produce a fatal bacteremia, a disease known as braxy or bradsot (367, 387). Braxy causes heavy mortality in populations of yearlings in Great Britain, Ireland, Norway, Iceland, and the Faroe Islands, although cases have been reported in Europe, Australia (107), the United States, and elsewhere. There are also numerous reports, published (367, 370) and anecdotal, of similar disease syndromes in calves (206).

The pathogenetic mechanism of the sudden multiplication and invasion of the abomasal lining in cases of braxy is not apparent, but ingestion of cold or frozen feed is frequently an associated factor in both sheep and dairy calves (367, 370). Icy feed material may impair mucosal function, allowing entry of the organism. *C. septicum* multiplies locally and disseminates throughout the body, producing local lesions and signs of toxemia. The walls of the abomasum and the proximal small intestine are edematous, hemorrhagic, and sometimes necrotic, while internal organs show only degenerative changes (107).

C. septicum produces numerous potentially or putatively toxic products, including α toxin (O_2-stable hemolysin [12, 14]), β toxin (DNase and leukocidin [71]), γ toxin (hyaluronidase [339]), δ toxin (O_2-labile hemolysin), a neuraminidase and hemagglutinin (130), a chitinase (76), weak lipase activity (149), and sialidase (450). In toto, the extracellular products of *C. septicum* cause increased capillary permeability and myone-

crosis, reportedly mediating spread of the infection (346). The systemic effects of the toxins and tissue breakdown products apparently result in a fatal toxemia in 2 to 3 days. With the exception of α toxin, none of these molecules has been purified to the extent that unambiguous statements can be made about their activities or potential roles in pathogenesis (163).

Recent work has provided definitive insights into the nature of the major toxin of *C. septicum*, α toxin (12, 14). It is an O_2-stable hemolysin with necrotizing and lethal properties (71); reports of lecithinase activity by α toxin are apparently erroneous. Purified α toxin, a cationic protein (pI, 8.4) of about 48 kDa, forms 230-kDa sodium dodecyl sulfate (SDS)-resistant aggregates (12). The 48-kDa molecule, an inactive protoxin, is activated by trypsin-mediated cleavage of a ≈4-kDa carboxy-terminal fragment (14). Activated toxin has a specific activity of $\approx 1.5 \times 10^6$ hemolytic units per mg, and the 50% mouse lethal dose is ≈10 μg/kg of body weight (12, 14). Toxin aggregated into 210-kDa complexes on erythrocyte membranes, and hemoglobin release from treated cells is preceded by release of potassium ions. Channels formed by activated toxin in planar membranes may be responsible for its cytolytic activity (14). Death following *C. septicum* challenge is delayed in α toxin-immunized mice. Protein antigenically compatible with α toxin was found in isolates of *C. septicum* but not in *C. perfringens*, *C. histolyticum*, *C. chauvoei*, or *C. difficile* (12). The gene for α toxin has been cloned (13, 183), and recombinant protein is hemolytic and lethal for mice. These findings provide a basis for definitive studies of α toxin structure-function relationships and for elucidation of its role in the pathogenesis of *C. septicum*-induced disease.

Prophylaxis and Therapy

Since the course of disease is rapid, prevention of *C. septicum*-induced infections is preferable to treatment, and commercial bacterins, toxoids, and bacterin-toxoids are available (376). Vaccines usually consist of inactivated liquid cultures, eliciting antibody responses to both bacterial surface antigens and toxic exoproducts (82, 150, 170). This vaccine usually produces lifelong immunity, although differences in immunogenicity by vaccine and by host species have been reported (143, 276). In one study of feedlot cattle, death losses were reduced by nearly 50% in vaccinates, with an estimated cost benefit of more than U.S. $10 per animal due to vaccination twice with a product that has an average cost of less than $0.25 per dose (217).

C. septicum is generally susceptible to penicillin G, ampicillin, chloramphenicol, clindamycin, cephaloridine, oleandomycin, erythromycin, lincomycin, and tetracycline (71, 138, 228, 379). Antibiotic susceptibility testing is often of little use because of the sporadic and rapidly fatal nature of disease caused by *C. septicum*. However, therapy can be useful in some situations, such as in outbreaks of gangrenous dermatitis in chickens (368).

Diagnosis

Diagnosis of *C. septicum* infection is relatively straightforward and is based on clinical signs, gross and microscopic findings postmortem, Gram stain of direct smears, and bacteriologic culture (69). If *C. septicum* predominates in the lesion or in pathological material obtained soon after the death of the animal, it is likely to be the etiologic agent. However, postmortem invasion from the gut by *C. septicum* is rapid, and in these cases, diagnosis is less certain. Infection by *C. chauvoei* should be ruled out in cases of myonecrosis (396). Many diagnosticians prefer to use a rapid method, such as fluorescent-antibody tests (20, 93, 165, 329). However, at the time that this was written, commercial production of labeled antisera had apparently ceased, presenting a potential challenge for veterinary diagnosticians. Diagnosis by direct gas chromatographic examination of affected tissues has also been described (216).

CLOSTRIDIUM DIFFICILE

Pseudomembranous colitis in humans results from overgrowth of the large bowel by *C. difficile*, usually after a perturbation in bowel flora, caused by antibiotic therapy or other circumstances (227, 347). An identical disease occurs in hamsters (74, 240) and guinea pigs (244) after antibiotic treatment. The organism has been isolated with a relatively high frequency from colonies of laboratory mice (184), a rate which can be augmented by antibiotic treatment. Strains of *C. difficile* that were fully toxigenic in vivo rapidly produce lesions in the cecum and colon of mice to a greater extent than in the small intestine (70). The organism causes hemorrhagic necrotizing enterocolitis in foals (202) and may cause chronic diarrhea in dogs. Although *C. difficile* has been reported as a cause of cecitis in rabbits (345) and hares (92), *C. spiroforme* is more common (38) (Table 2).

Playing a major role in the pathogenesis of *C. difficile* infections, apparently across host species, are two protein toxins, designated A and B (246). Immunization with both is required to protect hamsters against *C. difficile* infection (246). Toxin A, often referred to as enterotoxin, causes fluid accumulation in the gut by a mechanism that does not involve stimulation of cyclic AMP production; it is lethal when administered orally to hamsters, although toxin B, often referred to as cytotoxin, is not. Toxin B does not cause fluid accumulation under experimental conditions in the bowel, but exposure to picogram quantities causes cultured cells to round, detach from the surface of the culture vessel, and die. Both toxins are lethal to hamsters and mice when administered intraperitoneally (247). When produced in vivo, the toxins induce a severe, often fatal hemorrhagic ileitis or cecitis, with ulceration, formation of a pseudomembrane, and watery and bloody diarrhea.

C. difficile has been isolated from a variety of sources, including marine sediment (254), soil and sand (152), the hospital environment (279), feces of nondiarrheic humans (118, 395, 430), camels, horses, and donkeys (152), dogs and cats (up to 39% prevalence rate) (310, 348, 436), domestic birds, cattle, ducks, geese, seals, and snakes (238), the immediate environment of household pets and the human genital tract (153), and rarely from septicemias and pyogenic infections in humans (2, 117, 139, 239) and domestic animals (169). When ecological niches in the bowel are unfilled (as in neonates, infants, and gnotobiotes) or are emptied (as with antibiotic therapy), large populations of *C. difficile* can develop (238). Detection of *C. difficile* in domestic animals and the occurrence of cases of *C. difficile*-associated disease in the absence of antibiotic therapy suggest that circumstances other than antibiotic depression of normal flora can produce conditions allowing *C. difficile* to become established and multiply in the bowel (357, 358). The occurrence of frank disease is probably a result of the dose of *C. difficile*, its ability to compete for nutrients, the toxigenicity of the colonizing strain, perhaps its ability to adhere to colonic epithelium, the presence in the microenvironment of organisms that affect multiplication of *C. difficile* and toxin production or activity, and the susceptibility of the host (443).

The higher incidence of infection (not necessarily disease) in human infants than in adults and the clustering of cases by time and place suggest acquisition of the organism from the environment or some other source (310, 348). In fact, it is appar-

ently common for outbreaks of *C. difficile*-induced disease in humans to be caused by nosocomial strains acquired from the hospital environment or the hands of health care workers (443). Supporting this hypothesis is the fact that experimental reproduction of disease usually requires administration of both the organism and an antibiotic to which *C. difficile* is resistant. This is not unlike the hypothesized pattern with *C. spiroforme*, in which disruption of the normal microbial ecology of the cecum at weaning or following antibiotic therapy provides a platform for multiplication of the organism acquired from the environment.

Isolates of *C. difficile* from pets, veterinary clinics, humans, and hospitals were compared by restriction endonuclease analysis, and the results suggested that isolates from pets were similar to those from veterinary clinics, while those from humans were similar to those from hospitals. These findings do not establish a connection between colonization of companion animals and occurrence of disease in humans, but geographic variation may have confounded the results (310, 348), leaving open to question the role of domestic animals as a source of *C. difficile* for human infection.

C. difficile-induced disease has occurred in a variety of domestic and wild species, including a Kodiak bear (311), a rabbit (345), adult horses and foals (203, 204, 413), swine (201), a penguin (168), and captive ostriches (127).

C. difficile is putatively important as a cause of both diarrhea and fatal necrotizing enterocolitis in horses, a condition characterized by profuse watery diarrhea and dehydration in foals, occurring during the first week of life (203, 204, 413). Toxigenic *C. difficile* was isolated from the feces of less than 1% of 161 healthy foals and from 10% of diarrheic foals and diarrheic adult horses. Other intestinal pathogens were ruled out in most cases, and it was concluded that *C. difficile* was a legitimate cause of diarrhea in these animals (22). Affected foals typically display severe hemorrhagic necrotizing enterocolitis, with colic, weakness, and dehydration, and with large numbers of gram-positive rods lining the surface of necrotic villi (203–205). Death usually follows onset of clinical signs within 24 h (203–205). The lesions are similar to those encountered in *C. perfringens* type C infection. The disease has been experimentally produced by oral administration of *C. difficile* (203, 204). *C. difficile* has also been isolated sporadically from adult horses with colitis (413).

The organism and its cytotoxin have been demonstrated in the feces of dogs with chronic diarrhea. Pups are more commonly infected than adults, as determined by a study in which both point prevalence and incidence were determined during a 10-week period. Infection with different toxigenic phenotypes in the same litter suggested transient infection with different strains, and the overall incidence was nearly 100% during the 10-week study period. There was no observed pathology in neonatal dogs (327). Since the organism is shed in feces of normal dogs, its presence in dogs with chronic diarrhea is of uncertain significance (31).

C. difficile-induced disease is often treated with vancomycin (43). Artificial colonization with intestinal flora from hamsters or humans inhibits establishment of *C. difficile* in the hamster model (227), and feeding of a soy fiber diet as a preventive increases survival time in challenged hamsters by about one-third (126).

Cytotoxin can be detected either by a cell culture serum neutralization assay or by an enzyme immunoassay (122, 230). *Clostridium sordellii* antitoxin neutralizes the cytotoxic activity of *C. difficile.*

CLOSTRIDIUM SPIROFORME

C. spiroforme is the cause of iota enterotoxemia of rabbits and other laboratory rodents (38, 39, 46, 58–60, 63, 64, 154, 159) (Table 2). This organism has a distinct, loosely coiled spiral form when grown on blood agar (42). The coils consist of a uniform aggregation of numerous individual semicircular cells joined end to end (42). This spiral morphology is less common than a semicircular shape in organisms cultivated in vivo.

Isolates of *C. spiroforme* from rabbits with iota enterotoxemia produce a toxin that is neutralized by serum prepared against *C. perfringens* ι toxin (38). Toxin produced in vitro is lethal for mice and dermonecrotic for guinea pigs (38, 71). Enterotoxemia can be reproduced with filtrates of cecal contents from rabbits that have died of the disease (342). Nontoxigenic strains are rarely isolated from diseased animals (38), although toxigenic strains have been obtained from feces of healthy humans and from the ceca of healthy chickens and rabbits (38).

Diarrhea caused by *C. spiroforme* occurs spontaneously in weaned rabbits (213), although there is some evidence that destabilization of cecal microflora may be important in initiation of the disease (60, 234–236). Affected animals have high concentrations of spores of *C. spiroforme* in cecal contents (10^6 per g), and ι-like toxin can be detected as well. *C. spiroforme* is apparently not a normal inhabitant of the rabbit bowel but is rather acquired from the environment. Weaning and antibiotic treatment provide the proper conditions, since they are both accompanied by disruption of the bowel flora (60, 66, 213). Under appropriate conditions, diarrhea with perianal staining develops rapidly, and death ensues soon after. Gross lesions include an immensely dilated cecum with watery contents. Necrosis of the surface epithelium is accompanied by a pronounced inflammatory infiltration of the lamina propria. In many cases, poor hygiene (342), stress (321), and diet (151, 321, 322) also have a prominent influence.

Proteins with ADP-ribosyltransferase activity and ranging in molecular mass from 43 to 47 kDa have been purified from *C. spiroforme*. These proteins (now referred to as toxin component Ia) have no activity when administered to mice or applied to Vero cells; however, lethal and cytotoxic effects occur when proteins with transferase activity are mixed with a trypsin-activated protein (toxin component Ib), also obtained from cultures of *C. spiroforme*. Proteins with transferase activity were immunologically cross-reactive with the light chain of *C. perfringens* ι toxin and *C. difficile* proteins with ADP-ribosyltransferase activity (331). Thus, ι toxin is apparently a binary toxin, mediating two independent activities, ADP-ribosyltransferase (for component Ia) and binding and internalization (for component Ib). Recent work demonstrates that the target of the toxin's ADP-ribosylating activity is monomeric actin, which is trapped in the unpolymerized form, leading to destruction of the microfilament network (1). Degenerate oligonucleotide probes derived from the amino acid sequence of the components of the *C. spiroforme* toxin were used to screen a DNA library to clone the genes for *C. perfringens* ι toxin (325).

C. spiroforme infections are usually treated by administration of antibiotics and change in diet. The type strain is susceptible to chloramphenicol, clindamycin, erythromycin, penicillin G, and tetracycline (71). However, other published results (62) suggest that the most active therapeutics are metronidazole and penicillin G, while vancomycin, bacitracin, lincomycin, and erythromycin had relatively high MICs ($\geq$8 μg/ml).

Complete and lasting protection against intraperitoneal challenge with ι toxin can be achieved in weanling rabbits by

vaccination with a toxoid (106). Adults vaccinated with this preparation did not pass immunity to their young. Apparently, no immunoprophylactic products are available commercially.

The presence of semicircular gram-positive bacteria in the feces or cecal contents of affected rabbits is suggestive of *C. spiroforme* infection (178, 449), but definitive diagnosis is based on demonstration of iota-like toxin by a lethality assay in mice or a cytotoxicity assay in Vero cells (61, 398, 449). Differential centrifugation can be useful in obtaining material enriched for *C. spiroforme* from the intestinal contents of rabbits (178).

CLOSTRIDIUM COLINUM

C. colinum is the etiologic agent of acute or chronic ulcerative enteritis of chickens, quail, and other wild and domestic fowl, including young turkeys, grouse, partridge, and other game birds (29, 320, 366, 382) (Table 2). The condition, which is characterized by sudden onset and rapidly increasing mortality in a flock, was originally known as quail disease because of its frequent enzootic occurrence in bobwhite quail (*Colinus virginianus*), from which the causative organism derives its name (26). The G+C content is 43 mol%, significantly higher than that of many pathogenic clostridia (26, 71).

Quail disease was apparently first reported as a specific syndrome in the early part of this century (277). A *Clostridium*-like agent was associated with the condition in 1939 (17, 18), and the organism was recovered from quail infected with inoculum cultivated in the yolk sacs of chicken embryos (318, 319). The same organism was found in chickens and turkeys. It was later cultivated on solid media, allowing its full characterization and eventual naming (26–28).

The natural history of *C. colinum* and ulcerative enteritis has not been completely explained, although some facets seem certain. Natural infection likely occurs via the oral route (17, 18). Since infections can recur annually on some premises, survival of *C. colinum* in soil has been assumed. Quail with chronic disease can be carriers of infection (17). In chickens, ulcerative enteritis is often preceded by coccidiosis, aplastic anemia, stress, or infectious bursal disease (29, 90, 326). Experimental infection is readily produced in quail inoculated with at least 10^6 organisms (25), while chickens are much more resistant to *C. colinum* as a primary etiologic agent (90). Overcrowding and poor sanitation are predisposing factors for some game birds (233).

Birds infected with *C. colinum* are usually 1 to 3 months of age and may display no signs preceding a rapid and fatal clinical course. After oral entry, *C. colinum* becomes established in the terminal third of the intestine. Hemorrhagic enteritis occurs in the duodenum, with intestinal and cecal necrosis and ulceration. A diphtheritic membrane is sometimes present, and perforation can follow, with development of peritonitis and fibrin adhesions. Microscopic findings include desquamation of mucosal epithelium, edema of the intestinal wall, engorgement of local blood vessels, and a prominent infiltration of lymphocytes. Granulocytic infiltration occurs adjacent to ulcers, which appear as hemorrhagic foci involving the villi but extending into the submucosa. Many bacteria are present in necrotic tissue. From the intestine, *C. colinum* makes its way to the liver, putatively by portal circulation, producing diffuse centrilobular or pinpoint necrosis (27). Splenic congestion, hemorrhage, and necrosis may also occur, but lesions do not develop in other systems (29). Birds often die after 24 to 48 h, and in those with a clinical course longer than 7 days, emaciation and pectoral muscle atrophy occur. Mortality is frequently 100% in young quail, while in chickens, the average is about 10% (29), ranging upward to more than 30% (212, 222, 417). In a flock, the course of the disease is usually about 3 weeks, with peak mortality during the second week (27, 28).

Little is known about the virulence attributes of *C. colinum* or about the pathogenesis of ulcerative enteritis. It has been isolated only from lesions in fowl (29) and is reisolated from the livers of quail dying from experimentally induced ulcerative enteritis (25). No pathogenic effects have been observed following intramuscular inoculation into guinea pigs; infection of humans by *C. colinum* has apparently not been reported (29).

Filtrates of supernatant fluids from cultures of *C. colinum* are not toxic for mice (28), although a recent report of an unusual form of ulcerative enteritis suggests that lack of toxin production by this organism may not be a universal phenomenon (326). The signs, which invariably included nephrosis, suggested enterotoxemia, resembling some cases of necrotic enteritis (166, 314, 316), but histologic examination of tissues from affected birds revealed lesions consistent with ulcerative enteritis. Toxin production by this organism deserves further examination.

Ulcerative enteritis can be diagnosed by gross observation of typical lesions in the intestinal tract, liver, and spleen. This should be supported by demonstration of the agent by bacteriologic culture from the intestine or liver (29). Isolation from the gut is facilitated by use of tryptose phosphate agar with polymyxin B (25 μg/ml). *C. colinum* can be cultivated on tryptose phosphate agar with glucose, yeast extract, and 8% equine plasma, incubated under anaerobic conditions for 1 to 2 days at 35 to 42°C. Alpha-hemolysis is typical (71), although some isolates are beta-hemolytic (408). Oval subterminal spores are readily demonstrable in tissue but are rare in artificial media. *C. colinum* most closely resembles *C. difficile* but can be distinguished from it on the basis of differences in gelatin hydrolysis and raffinose fermentation (386). Stained impression smears are also useful in diagnosis (29), and a fluorescent-antibody test has been developed (24).

C. colinum is usually susceptible in vitro to tetracyclines, chloramphenicol, clindamycin, erythromycin, penicillin G, and bacitracin (29, 71). Streptomycin is apparently effective in vivo (320), although the organism is resistant in vitro. Bacitracin and streptomycin are most commonly used as therapeutics, although bacitracin-resistant strains of *C. colinum* have been reported (369).

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